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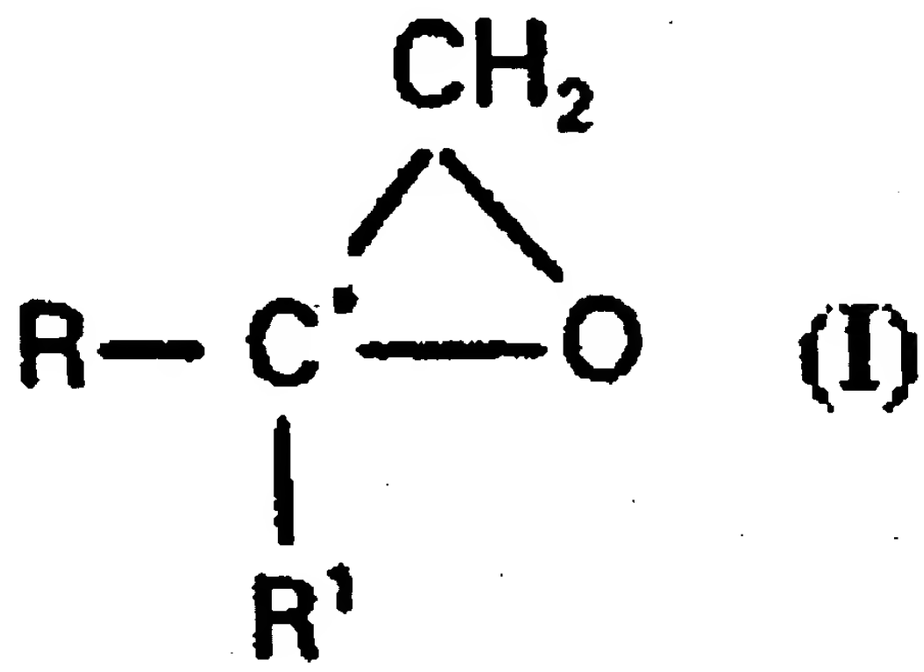

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CHIRAL SYNTHESIS OF TERTIARY ALCOHOLS WITH A HYDROLASE

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Abstract: Method of preparing an optically active compound of formula (I), wherein R and R1 are independently alkyl, alkenyl, alkynyl, alkoxy, alkoxyalkyl, cycloalkyl, aryl, aralkyl, a heterocyclic group or a C1-C4 alkyl-heterocycle, each being optionally substituted, provided that R and R1 are not identical and * is an optically active chiral centre, from the corresponding racemic ester or diol by treating with a hydrolase enzyme.

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Detailed Description

CHIRAL SYNTHESIS OF TERTIARY ALCOHOLS WITH A HYDROLASE.

I This invention relates to a method of chiral resolution of tertiary KKK and to novel compounds useful in the method.

Certain tertiary alcohols are useful compounds in pharmaceutical and agrochernical oudetn for example, the compounds disclosed in GB 1529818, EPT-1 5756, EPT-44605, EP-13-61835, EP-13-1 31684, EP-A- 47594, GB 2064520 and EPA-47239T T'hese compounds usually have an optically active chiral centre and resolution of the compounds can lead to benefits for example, greater activity or lower toxicity with one of the optically active isomers.

European patent appl'ication EPA-472392 discloses the compound (+)-2-(2,4-difluorophenyl)-l -[3-[(E)-4-(2,2,3,3- tetrafluoropropoxy)styryll- 1H-1,2,4-triazol-1-yll-3-(lH-1,2,4-triazol-1-yl)propan-2-ol, which has antifungal activity, and is valuable in treatment of fungal infections in man and in other animals. Intermediates for the preparation of the compound are also disclosed.

EPA472392 discloses two methods for the preparation of an optically active epoxide of formula (A) F CH2 C*- 0 F CH2 OH (A) The first method is a chemical synthesis as demonstrated by processes 1 to 4 of EPA-472392. Process 4 is an asymmetric oxidation process of 2-(2,4-difluorophenynaHyl alcohol with t-butyl hydroperoxide in organic solvents such as methylene chloride in the presence of a titanium tetrabkoAdp such as thaniurn tetra i sopropoxide or titanium tetrabutoxide and of a dial kyl-ta rtrate.

The second method is demonstrated by processes (i) to (iv) of EPA-472392. Process (iii) involves an enzymatic asymmetric ester hydrolysis of 1-acetoxy-2-(2,4-difluorophenyl-2,3-epoxypropane performed using 6 hydrolytic enzyme such as an esterase or a lipase in a buffer solution or in a mixture of buffer solution and an organic' solvent such as diisopropyl ether, ethanol, acetone or dimethy1formamide. In this process the (+)-form undergoes ester hydrolysis selectively and (+)-2- (2,4-difluorophenyl)-2,3-epoxypropanoI is obtained. The (-)-l-acetoxy-2- (2,4-difluorophenyl)-2,3-epoxypropane does not Undergo essr hydrolysis and is recovered from the reaction liquid in high optical purity. In process Ov) the (-)-l-acetoxy-2-(2,4-difluorophenyl)-2,3-epoxypropane recovered from process (iii) undergoes a normal ester hydrolysis using bases such as potass[urn hydroxWe or sodium hydroxide and (+2-(2,4-difluorophenyl)-2,3-epoxypropanoI is obtained.

Both the (+) and H forms of 2-(2,4-difiuorophenyI)-2,3- epoxypropanol can be converted to (+)-2-(2,4-difluorophenyl)-l-[3-[(E)-4- (2,2,3,3-tetrafluoropropoxy)styryll-1 H-1,2,4-triazol-1 -yll-3-(l H-1,2, 4-triazol-1-yl)propan-2-ol involving different methods. The disadvantage of this method is that the epoxWe is a reactive group which is susceptible to hydrolysis and nucleophilic attack, for example by an amino group on the enzyme. This would result in the enzyme being completely or partially deactivated.

The present inventors have found that the process can be improved by enzyme catalysed resolution of an ester or diol followed by chemical steps to the corresponding resolved epoxide.

Thus, according to a first aspect of the present invention there is provided a method of preparing an optically active compound of formula CH2 R - C*- 0 R1 wherein R and R1 are independently alkyl, alkenyl, alkynyl, alkoxy, alkoxyalkyl, cycloalkyl, aryl, aralkyl, a heterocyclic group or a Cj-C,' alkyl-heterocycle, each being optionally substituted, provided that R and R1 are not identical and * is an optically active chiral centre; the method comprising (a) treating a racernic compound of formula (11) 0

CH2-0-C-R 2 R- C-OH R1 0 1) wherein R and R1 are as previously defined and R 2 is alkyl, aryl or aralkyl each optionally substituted, with a hydrolase; or (b) treating a racemic compound of formula (111) CH20H R - C-OH R1 wherein R and R' are as previously defined, with a hydrolase in the presence of an acyl donoq and converting the optically active products of (a) and/or (b) to the optically active compound of formula (1).

When either of R and R' is aryl it is preferably phenyl or substituted phenyl.

When either of R and R' is aralkyl it is preferably benzyl or substituted benzyl.

When either of R or R' is cycloalkyl, it is preferably C3.7 cycloalky(, particularly cyclopropyl, cyclopentyl or cyclohexyl.

When either of R or R' is alkyl, alkenyl, alkynyl, alkoxy or alkoxyalkyl it preferably contains up to 10 carbons, especially 1 to 6 carbon atoms.

When either of R or R1 is a heterocylic group or -Cl-C4 alkyl- heterocycle, the heterocycle is preferably selected from 1,2,4-triazole, 1,3,4-triazole, imidazole, pyrimidine, pyrazine, oxazole or pyrazole. The heterocycle may be substituted, for example, with amino, oxygen, S02R 4 or OR4 where R is hydrogen C,-6 alkyl or phenyl. When RI is -CI-C4 alkylheterocycle, the alkyl group is preferably methyl or ethyl.

An especially preferred heterocycle is 1,2,4-triazole and imidazoie.

Preferably, R is an optionally substituted aryl or optionally substituted aralkyl group and R' is an alkyl, alkenyl, alkynyl, alkoxy, alkoxyalkyl, cycloalkyl, aryl, aralkyl, a heterocyclic group or a C,-C, alkyl-heterocycle, each being optionally substituted, provided that R and R' are not identical.

When either or both of R and R' is substituted the substituent is preferably selected from: halogen, preferably chlorine, fluorine or bromine; alkyl, preferably C,-,, alkyl, especially methyl, ethyl, propyl, and butyl (n-, iso-, sec and tertiary forms thereof); alkoxy, preferably Cl., alkoxy, especially methoxy, ethoxy, propoxy and butoxy; haloalkyl, preferably C`1.6 haloalkyl especially substituted with chlorine or fluorine, particlularly trifluoromethyl and pentafluoroethyl; haloalkoxy, preferably C,.6 haloalkoxy especially substituted with chlorine or fluorine, particularly trifluoromethoxy; cyano; nitro; amino; hydroxy; sulphonyl; and phenyl.

Them may be one or more substituents and preferably when substituted there may be 1 to 3 substituents.

An especially preferred R group is phenyl substituted with one or more halogens, particularly 4-chloro, 4-fluoro, 2,4-dichloro and 2,4- difluorophenyl.

The hydrolase enzyme may be lipase, esterase, phosphatase, amidase, peptidase, sulphatase, nitrilase or glycosidase. The enzyme may be obtained from microbial culture or from plants or animals.

Preferred examples of such enzymes are pig pancreatic lipase and lipase from Chromobacterium viscosum. Such enzymes are commercially available or can be prepared by methods known in the art.

The enzyme catalysed resolution can be carried out by any one of the following routes :

(1) hydrolytic reaction in an aqueous reaction medium; (2) hydrolytic reaction in an aqueous medium with a miscible organic solvent; (3) hydrolytic reaction in an aqueous/organic 2-phase reaction medium; (4) esterification reaction in an organic reaction medium; (5) transesterification reaction in an organic reaction medium.

It will be appreciated that because of the rules of nomenclature of optical forms, the designation of the configuration of the epoxide depends on the nature of the substituents about the asymmetric centre.

In the general and specific examples given below the two forms of the epoxide are (R)-(+) and (S)-(-) while the two forms of the diol and ester are (R)-(-) and (S)-(+). It will be further appreciated that the system illustrated by the specific examples behvv can provide both configurations of the epoAde by selechon of the appropriate ester or diol.

The method of the present invention when carried out by any of routes (1) to (3).is summarised by the following general reaction sequence: 0 CH2 CH20H CH2-0-C-R 2 R-C-0 K-C-OH R-C-011 K' to R' M 0 R1 01) Step (i) Step (ii) Step (iii) 0 CH2 CH20H CH2-0-C-R 2 R- C*-O R- C*-OH R - K R1 Step (v) Step (iv) in which the compounds of formula (11) and (111) are racemic mixtures of (R)-(-) and (S)-(+) enantiomers. The racemic mixture of the compound of formula (11) is treated with a hydrolase, for example pig pancreatic lipase.

The hydrolase spedfically converts the undesired (S)-(+) ester to the corresponding (S)-(+) diol of formula (111). A small amount of (R)-(-) diol of formula (111) may also be formed. This conversion leaves the (R)-(-) ester of formula (11) free from the undesired (SPW ever of formula 00.

The (S)-(+) diol of formula (111) can be separated and ether discarded, or Overted or partially inverted to give the enriched (R)-(-) diol of formula (111) or racernised to give racemic diol.

Reaction sequences (1) to (3) are more specifically illustrated by using the triazole epoxide intermediate for (+)-2-(2,4-difluorophenyl)-l- [3-[(E)-4-(2,2,3,3-tetrafluoro-propoxy)styryll-1 H-1 2,4-triazol-1 -yll-3-(l H- 1,2,4-triazol-1-yf)propan-2-ol and 2-(2,4-difluorophenyl)-1,3-di(1,2,4- triazol-1-yl)-2-propanol (pharmaceutical fungicide, common name fluconazole):

0 F CH2 F CH20H F CH2-O-C-C3H7 C- 0 L;-Utl U -,-Ijrl F Ut'2 F 2 F CH2 N-N N N N N N Step (i) LNJ Step (ii) ~ NJ Step (iii) If 0 11 F CH2 F CH20H F CH2-0-C-C3H7 C*- 0 U- UM UM F k' r' 2 F ~'n2 IF CH2 I I I N-N N-N N-N N 'J Step M Step Ov) L NJ The method of the present invention when carried out by (4) and (5) is summarised by the following general reaction sequence :

CH2 CH20H R-C-0 R-C-01-1 I I I h, (11 N' till) Step (a) Step (b) CH2 CH20H R-C*- 0 R- R, Step (c) in which the compound of formula (111) is a racemic mixture of (R)-(-) and (S)-(+) enantiomers. The racemic mixture is treated with a hydrolase, for example pig pancreatic lipase, in the presence of an acyl donor, such as an alkenyl ester, for example vinyl acylate, particularly vinyl acetate, vinyl propionate, vinyl butyrate or vinyl benzoate, or such as isopropenyl acetate or isopropenyl butyrate, or an alkyl ester such as ethyl acetate or an acid of the formula RC02H where R is alkyl, preferably C1-6 alkyl, for example, acnic acid, propanoic acid and butyric acid.

The hydrolase specifically converts the undesired (S)-(+) diol to the corresponding (S)-(+) ester of formula (11). A small amount of (R)-(-) ester of formula (11) may also be formed. This conversion leaves the (R)- (-) diol of formula (111) free from the undesired (S)-(+) diol of formula (111).

The ester of formula (11) can be separated and either discarded, or hydrolysed and inverted or partially inverted or racernised to give the (R)- diol or racemic diol of formula (11).

Reaction sequences (4) and (5) are more specifically illustrated using the triazole epoxide intermediate for 2-(2,4-dichlorophenyl)-l -(1 H- 1,2,4-triazol-1-yi]-hexan-2-ol (agricultural fungicide, common name hexaconazole):

Cl CH2 Step (a) ClCH20H 0 L;-UH Cl Cl UM2 N-N N-N N J Step (b) Cl CH2 Step (c) Cl CH20H C%-- 0 C ~- 0 H Cl Un2 Cl Ut'2 N N N-N Li Nl:;" ~1 N J Thus, the enzyme catalysed reaction can be

operated in both the hydrolytic direction as described above and also in the esterification direction by reacOng the racernic diol of formula (111) with an acyl donor, such as an alkenyl ester, for example vinyl acylate, vinyl acetate, vinyl propionate, vinyl butyrate or Wyl benzoate, or such as isopropenyl acetate or isopropenyl butyrate, or an alkyl ester such as ethyl acetate or an acid of formula RC02H where R is alkyl preferably C,-, alkyl, for example, acetic acid, propanoic acid, butyric acid, in the presence of a hydrolase enzyme. This transesterification route provides the ester and diol of the opposite configuration to that obtained when that same enzyme is used to catalyse the reaction by the hydrolytic route.

C- 'C H 2 ZN, In all the reactions described above it is possible to recycle the unwanted enantiomer to give much greater yields and thereby gain significant economic advantages. In the hydrolytic reactions the (S)- diol is recycled to the racernic W and in the transesterification reactions the (S)-ester is recycled to the racemic diol.

The (S)-ester may be recycled by adding an aqueous base such as sodium hydroxide to hydrolyse the (S)-ester to (S)-diol. Optionally the diol may be isolated at this stage by cooling the solution to crystallise out the product or may be kept in solution in aqueous base. In both cases the (S)-diol may be reamed with an oplonally substituted aryl or alkyl sulfonyl halide, for example p-toluene sulfonyl chloride, p-nitrophenyl sulphonyl chloride, p-bromophenyl sulfonyl chloride, methansulfonyl chloride or trifluoromethansulfonyl chloride, optionally in the presence of a phase transfer catalyst which is preferably an alkylammonium salt such as tetr6butyl ammonium bromide. The product of the reaction is the compound of formula (1) which is substantially the (S)-enantiomer. The product is formed via an intermediate sulfone which may be optionally isolated depending on the concentration of the aqueous base used.

The (SPepokde (formula (SPOA is hydrolysed with an aqueous acid at a temperature of between 0 and 100'C, preferably 101C to 800C and especially from 20*C to 700C. The product is the diol of formula (111) composed of mixtures of (R) and (S) enantiomers, the ratio of which depends on the concentration of aqueous acid used, the temperature of the hydrolysis reaction and the reaction time. For example, at a temperature of 251C and a reaction time of 24 hours using 1.5M sulfuric acid the percentage of (R) to (S) enantiomers in the product was 73.5% and 26.516 respenively. At a temperature of 651C and reaction time of 20 minutes using 8.3M sulfuric acid the percentage of (R) to (S) enantiomers in the product was 53% and 47% respectively.

The racemic diol may be isolated by basidifying the acidic solution, extracting the diol into an organic solvent such as toluene and crystallising the diol by concentrating and cooling the solution.

The (S)-diol may be recycled by reacting with an aryl or alkyl sulphonyl halide such as p-toluenesulfonyl chloride, p-nitrophenyl- sulfonyl chloride, p-bromophenylsulfonyl chloride, methansulfonyl chloride or trifluoromethansulfonyl chloride, in a solvent such as toluene, xylene, benzene, pyridine, rn ethyl isobutyl ketone or tetrahydrofuran with a base such as an alkylamine, preferably triethylamine or pyridine or N,N- dimethylaminopyridine or an aqueous base such as sodium or potassium hydroxide. Depending on the stoichiometry of base used the (S)-1 - sulfonylester may be formed or with excess base the (S)-epoxide of formula (1). Preferably excess base is used and the (S)-epoxide is formed.

When aqueous sodium hydroxide is used as a base a phase transfer catalyst such as tetrabutylammonium bromide may be used to accelerate the reaction. Suitable temperatures are between -51C and 500C, preferably 200C to 450C, and reaction times of 0.25 to 24 hours, preferably 0.5 to 1 hour. The (S)-epoxide is then treated as previously described for the (S)-epoxide from the recycling of the (S)-ester.

The general conditions for the enzyme catalysed reactions (1) to (3) are conventional. The enzymes are commercially available and used in the reaction mixture as received with no further treatment or the

enzymes can be prior treated by dissolving in buffer at around pH7 to 7. 5 and adsorbing to a support, for example, passively adsorbing to glass beads, sand, diatomateous earths (eq. WWI charcoal, aWmina AnOy, silica gel, kieselguhr or resins, e.g. Amberlite*, Dowex*, XAD* resins. Alternately the enzymes can be covalently adsorbed to a support, for example, polystyrene, epoxy resins such as Eupergit*, plastic supports.

indicates a trademark or tradename. The adsorbed enzyme may be dried by Iyophifisation (freeze-drying) of the water and then ground to a homogenous powder.

A suitable buffer is used to maintain an appropriate pH, preferably pH 5 to 9, especially pH 7.5. An aqueous base such as sodOm or potassium hydroxide may optionally be added to maintain the desired pH.

The reaction mixture must be stirrel The temperature of the reaction mixture is suitably from 1 50C to 350C and preferably from 300C to 350C.

The solvent for the hydrolytic reaction is water, preferably with a miscible co-solvent such as an alcohol, preferably methanol or Wanol, or an amide, preferably dimethyl formamide, or dimethyl acetamide or a sulphoxide, preferably dimethylsulphoxide, or a nitrile, preferably acetonitrile, or an ether, preferably tetrahydrofuran or 1,4-dioxan or a ketone, preferably acetone or 2-butanone. Alternately an immiscible co- solvent may be used, for example, an arene, preferably toluene, xylene or benzene, an ether preferably t-butylmethyl ether or diethylether or a ketone, preferably 2-pentanone, 3-pentanone, 2-hexanone or 3-hexanone.

In a further alternative, mixtures of water miscible and immiscible sokents may be used preferably toluene and methanol, xylene and ethanol, t-butylmethylether and methanol, 2-pentanone and acetone. An especially preferred mixture of solvents is water, methanol and toluene.

The amount of miscible co-solvent is small enough to maintain both the active enzyme and a biphasic mixture but is large enough to solubilise the reactant ester.

Solvents for the transesterification reaction are suitably tetrahydrofuran, tertiary-butanol or short chain ketones such as methyl isopropyl ketone, methyl ethyl ketone (2-butanone), methyl propyl ketone (2-pentanone), methylisobutyl ketone, or toluene or a vinylacylate, e.g. vinyl acetate.

For the transesterification reaction the solvent is preferably anhydrous, Le the solvent contains 0.5% or less water, preferably 0.1 % or less water, especially 0.05% or less water (% by weight).

In situations where there is 0.5% or less water in the solvent it is known in the art to buffer the solvent at a particular activity of water which is less than that at 0.5% water by adding a hydrated or anhydrous salt or mixtures thereof. The effect is to maintain a lower activity of the water in the solvent.

It is preferable to pass nitrogen gas through the transesterification reaction system (step b) which leads to a 3 fold increase in the reaction rate. This means the reaction time is decreased or that less enzyme can be used, for example it has been demonstrated that 25% less enzyme can be use& This represents a significant economic advantage. Thus, in a preferred aspect of the present invention there is provided a method according as previously described above wherein the diol of formula (111) is treated with a hydrolase in the presence of a flow of nitrogen containing gas.

Although the transesterification reaction can be carried out at relatively low temperatures, for example 301C, it has been found advantageous to raise the temperature to 501C to 80*C, preferably 601C to 750C, especially 651C to 701C. Thus the transesteribcabon reaction may, be! carded out at tempemtwes ranging from 300C to 801C.

When the transesterification reaction has run to completion (i.e.

the (R)-diol is of high optical purity) the'(R)-diol may be conveniently isolated by filtering off the enzyme and either concentrating the organic solution and seeding with (R)-diol to selectively crystallise the (R)- diol from the mixture or by removing the organic solvent and triturating the resultant oil with a different organic solvent, preferably dichloromethane, from which the R-diol crystallises out from the mixture. The optical purity of the (R)-diol may be enhanced during this process.

Solvents for the extraction of the product of the enzyme catalysed hydrolytic reaction routes (1-3) include any aqueous immiscible solvent, for example, alkane such as hexane or octane, ether or ketone solvents such as tertiary-butyl methyl ether, 2-hexanone, diethyl ether, pentanone or methyl isobut" ketone, arene solvents such as toluene, x"ene or benzene. Particularly preferred solvents are toluene and m ethyl isobutyl ketone.

Further, recrystallisation of the product from organic solvents such as ethyl acetate, ether, hexane, dichbromethane or solvent mixtures thereof, results in the optically pure substance. Especially preferred is dichloromethane since this solvent enhances the optical purity of the product particularly from the transesterification reaction, i.e. it is possible to obtain 99.9% enantiomeric excess using this solvent.

The racemic form of the triazole epoxide can be prepared by the methods described in EP 44605 which involve reacting the corresponding ketone with trim ethyl oxosu lphonium iodide (Corey and Chaykovsky, JACS, 1965, 87, 13511364) or trimethylsulphonium iodide (Corey and Chaykovsky, JACS, 84,3782) using methods set out in the literature.

The ketone can be made by methods set out in the patent literature, more particularly in British Patent Specifications Nos. 1533705 and 1533706.

The racemic epoxide of formula (1) can be hydrolysed with dilute acid, for example sulphuric acid, to produce the racemic diol of formula (111). Treatment of the racerric diol of formula (111) with an acid chloride results in the esterification of the primary alcohol to yield the corresponding racemic ester of formula (11).

The racemic and enantiomeric forms of the triazole substituted ester of the present invention are novel, thus according to a further aspect of the present invention there is provided a compound of formula 0 0 2 CHrO-C-R I & -OH CH2 I N-N LNJ and enantiomeric forms thereof, wherein R 2 is alkyl or aryl, both optionally substituted, X is halogen, alkyl, haloalkyl, alkoxy, halo'alkoxy, cyano, nitro, amino, hydroxy, sulphonyl or phenyl; and n is 0 to 5.

Preferably R 2iS Cl.r, alkyl, especially methyl, ethyl, propyl and butyl, (n-, iso-, sec- and tertiary forms thereof).

When X is halogen it is preferably chlorine, fluorine, bromine.

When X is C1.6 alkyl, it is especially methyl, ethyl, propyl or butyl, (n-, iso-, sec- and tertiary forms thereof); or C,.6 alkoxy, it is especially methoxy, ethoxy, propoxy and butoxy; or C1-6 haloalkyl, the halogen is preferably chlorine or fluorine and is especially trifluoromethyl or pentafluoroethyl; or C1. haloalkoxy, the hahgen is preferably Worine or fluorine and is especially trifluoromethoxy.

n is preferably 0 to 3.

The enantiomeric epoxide intermediates of the present invention are useful intermediates particularly in the preparation of agrochemicals and pharmaceuticals.

For example, the (R)-(-) diol of formula (111) where R is 2,4- difluorophenyl and R1 is -CH2-1,2,4-triazole can be converted to the corresponding (Rb(+) epoxide of formula (1) by the method of process 6 in EPA-472392.

The (R)-(+) epoxide of formula (1) when R is 2,4-difluorophenyl and RI is -CH2-1,2,4-triazole can be used to make (+)-2-(2,4- difluorophenyl)-l-[3-[(E)-4-(2,2,3,3-tetrafluoro-propoxy)styryll-lH-1,2, 4-triazol-1 -yll-3-(l H- 1, 2,4-triazol- 1 -yl)propan-2-ol according to the method of process 7 disclosed in EPA-472392.

Thus, in a further aspect of the present invention there is provided a method for preparation of compounds of formula V:

OH C*- R 5 CH2 N-N Y -'~N J M wherein R' is any of the groups previously defined for R, Y is an optional substhuent 0: is halogen and n is 0 to 3, which c W preparing a compound of formula (IV):

CH2 x" C* 0 CH2 N N (IV) by (a) treating a compound of formula 0 CI-12-0-C-CiH7 I X C- 0 IH Z.1!.1 I I trrc H 2 1 N - IN U with a hydrolase; or (b) treating a compound of formula CH20H U-UH CH2 N-N U with a hydrolase in the presence of an acyl donor; (c) converting the optically active products of (a) and (b) to the optically active epoxide of formula (IV); (ii) converting the optically active epoxide of formula (IV) to the optically active compound of formula (V) in an organic solvent such as teuahydrofuran, dimethy1formamide or ethanol, in the presence of a base such as potassium carbonate, potassium bicarbonate or sodium hydride.

R' is preferably alkyl, aryl, heterocycle or alkyl-heterocycle as defined above for R and R1, X is preferably chlorine or fluorine and Y is an optional substituent. Of particular interest are the compounds where X is 2,4-difluoro, 2,4-dichloro, 4-chloro and 4-fluoro, R' is C,.4 alkyl, phenyl or halophenyl (particularly 2,4-difluoro, 2,4-dichloro, 4-chloro and 4- fluoro phenyl), 1,2,4-triazole or -CH2-1,2,4-triazole each optionally substituted as previously defined and Y is an optional substituent which may be the same as defined above for R or may be more complex such as the 2,2,3,3-tetrafluoro-propoxy)styryI substituent mentioned above.

The invention is further illustrated by reference to the following examples which do not limit the scope of the invention.

EXAMPLE 1 lRyparation of (RLLL2&vdroxy-2-(2,4-difluorophenyl)-3-(lH-1,2,4-triazol- 1-v0propylbutyrate by hydrolytic resolution of the corresponding racemic ester in an aqueous reaction medium.

Tris-hydrochloride buffer (10mM, pH7.2, 8.334 litres) was placed in a glass reactor and heated to 350C with sti"ing. To this was added fig pancreatic Qase (50 BiocatalVsts, Treforest, UK) and the pFH adjusted to pH 7.2 using sodium hydroxide (2M).

The corresponding racemic ester of formula (1) where R 2 = C31-17 (1 40g) was dissolved in methanol (1.666 litres). The reaction was started by addition of methanol solution to the aqueous enzyme solution.

The substrate was added by pumping at a rate of 67ml/minute. The reaction pH was monitored and automatically adjusted to pH 7.2 by titration with sodium hydroxide (M.

After 3 hours the reachon had ceased, no further titration was observed. The reaction mixture was extracted twice with 2.5 litres of toluene. The toluene was dried with anhydrous sodium sulphate and the toluene removed by vacuum distillation. The residual aqueous phase was extracted twice with 2.5 litres of ethyl acetate, dried with anhydrous sodium sulphate and the solvent removed by vacuum distillation.

The reaction was monitored by chiral stationary phase HPLC under the following conditions :

column Chiralcel O.D. (250mm x 4.6mm) eluant hexane:2-propanol (87.5:12.5) how rate 0.6ml/minute detector UV absorbance (205nm) retention times (S)-(+)- ester - 20 minutes (R)-(-)- ester 24 minutes (R)-(-)- diol 28 minutes (S)-(+)- diol - 36 minutes The combined toluene extracts yielded 58.7g of an oil of the following composition : (R)-(-) ester - 58.01g (S)-(+) ester - not detectable (S)-(+) diol - 0.69g (R)-(-) diol - not detectable EXAMPLE 2 Preparation of (RLLL2&#0000;The corresponding racemic ester of formula (1) where R 2 = C3H7 (44kg) was dissolved in a mixture of methanol (25 litres) and toluene (125 litres) bV heating to 600C. The solution was then cooled to 350C.

Aqueous potassium phosphate buffer (50m M, pH 7.5, 260 litres) was charged to the reactor and heated to 3 5 + /-1 1 C. To this was added pig pancreatic lipase (1.5kg, previousIV dissolved in 10 litres of phosphate buffer, pH 7.5) aind the reactor stirred. Toluene (50 litres was then added to the reactor and the reaction was initiated bV pumping the toluene/methanol mixture into the aqueous enzVme solution at a rate of 3.3 litres/minute. The reaction mixture was maintained at pH 7.5 + /-0. 2 Q the addition of 1 molar sodium hVdroxide. The reaction was monhored Q chiral stationarV phase IAPLC under the condkions described in Example 1.

The reaction was complete after ?5 hours lindicated Q the residut Nb(+)- ever being present at less than 1 %). Celite (5kg) was stirred into the reaction mixture and the reaction mixture was passed through a filter cloth to remove protein and aid phase separation. The aqueous and organic laVers were separated. The organic laVer was washed three times with 1000 litres of warm (35 OC) water to remove anV diol. The final organic solution contained only 30bester, the Vield being 1 5kg and 98 % enantiomeric excess.

The aqueous phase was extracted three times with ethVI acetate (65 litres). The combined ethVI acetate extracts contained 1 5kg of (S)- (+)-diol of 80% enantiomeric excess.

This example was repeated using pig pancreatic lipase or lipolase (obtained from Novo ~ndustri) as the enzyme. The ester (100mg) was dissolved in 7.5ml of t-butyl methVI ether and reacted with the enzyme in 30 ml of aqueous buffer as described above. The results are presented in Table 1.

A bWl e I Enzyme Enantiomeric excess of (R)-(-)-diol M pig pancreatic lipase 96 Qdase 20 EXAMPLE 3 heyantion of (R)-(-)-2-(2,4-difluorophenyl)-3-(lH-1,2,4-triazol-l- YI)r)ropan-1 2-diol by hydrolase catalysed transesterfication in various organic solvents.

The reaction mixtures consisted of the corresponding racemic diol of formula (111), 25mg; enzyme, 5 mg; and solvent 2ml. The reactants formed a slurry in the solvent due to the insolubility of the enzyme and the paqkl solubility of the diol substrate. The reaction mixtures were incubated with shaking at 37 0 C for 16 hours. Enzyme was removed by filtration through a membrane filter (0.2 micron diameter pores). Where the substrate or product was incompletely soluble in the reaction solvent, methanol (0.25ml) was added to the mixture prior to filtration. This resulted in a clear solution from which the suspended enzmye could be removed by filtration. Filtrates were analysed by HPLC as described in I Example 1.

Table 11 shows the results of the transesterification reaction with a range of hpases using vinyl butyrate as the solvent and acyl donor.

Table 11 Enzyme Enantiomeric excess of (R)-(-)-diol M Chromobacterium Miscosurn 94 Pig pancreatic lipase 51

The method of Example 3 was repeated using various solvents and the enzyme Chromobacterium viscosum lipase. The reaction mixture contained: solvent 2ml, vinyl butyrate O.1ml, diol 25 mg, enzyme 5 mg.

The reaction mixture was incubated with shaking at 371C for 16 hours.

The results are presented in Table 111.

T a bWl e IN I Solvent Enanhome6c excess of (R)-(-)-diol (%) 2-pentanone 81 butanbne 77 tetra hydrof ura n 71 PCT/GB94/00793 23 tertiary-butanol 41 Analytical methods for the following examples were as follows: GLC was carried out using a Hewlett Packard HP~890 and integrator with a 0.25mm capillary, 25mm CP,Sil-5CB (Chrompack). With a temperature/tim6 profile of 1400C for 2 minutes then 100C rise per minute to 2000C and then held at this temperature for 5 minutes. The diol elutes at about 3.8 minutes and the ester at about 5 minutes.

Reverse phase HPLC was cauied out using a Hewlett Packard and integrator with an ODS 25cm x 4.6mm ID analytical column and eluent of 47.5% acetonitrile and 52.5% water at a flow rate of 1m./min.

Detection was by ultraviolet at 260nm.

Chiral HPLC was carried out using a Hewlett Packard and integrator with a Chiralcel OD25cm x 4.6mm ID analytical column and eluent of 10% absolute ethanol and 90% n-hexane at a flow rate of lml/min. The elution times were approximately as follows: (S)-ester 14.4, (R)-ester 15.8, (R)-diol 20.7, (S)-diol 23.4 minutes.

I EXAMPLE 4 1 weight equivalent of racemic diol, 2-(2,4-difluoro -phenyl)-3-0 H-1,2, 4- triazol-1-yl) propan-1,2-diol was dissolved in 20 volume equivalents of methyl isobutylketone (MIBK) at 60-650C and the mixture was stirred. 4 volume equivalents vinyl acetate were added and then a stream of nitrogen sparged through the solution via a sintered glass lance. 0.5 weight equivalents of pig pancreadc lQase (obtained from Biocatalysts) was added in one portion. The reaction was monitored by GLC, method given above, and towards the end of reaction Chiral HPLC, method given above. The reaction was stirred for 13 hours untH less than 516 A-diol remained. The enzyme was filtered from the hot solution using a sintered glass filter and washed with a small amount of cold MlBK. The solvent was evaporated under reduced pressure on a rotary evaporator, and the resulting oil triturated with 2 volume equivalents of dichloromethane.

After cooling to 50C and standing for thirty minutes the white crystals of R-diol that had formed were filtered off, washed with 2 volume equivalents of cold dichloromethane and air dried. This procedure gave 0.44 weight equivalents of (R)-(-)-2-(2,4-difluorophenyl)-3-(1 H-1,2,4- triazol-1 -yl) propan-1,2-diol, which by Chiral HPLC gave greater than 99.5% of the (R)-enantiomer. This repesents a 42% conversion.

The filtrate contained 0.8 weight equivalents of a mixture containing 75% (S)-ester, 7.5% (R)-ester, 7.4% (R)-Diol and 8.1 % (S)-Diol.

EXAM LE 5 1 weight equivalent of raceadc diol (formula 111) was dissolved in 5 volume equivalents of MlBK at 700C. Nitrogen was sparged through the solution and 2.5 volume equivalents vinyl acetate were added, followed by 0.25 weight equivalents of pig pancreatic lipase (obtained fro Biocatalysts). The mixtum was stirred at 701C for 6-7 hours or < 5 % (S)-diol remained by chiral HPLC. The solution was filtered washed with 2 volume equivalents of warm MlBK. The solution concentrated to one-third volume and added dichloromethane anc with (R)-diol. The crystals were filtered off and air dried. This pr yielded 0.44 weight equivalents of materials of greater than 99.5 diol and greater than 85-90% strength (dichloromethane was fou approximately 10-15% of the crystal mass).

EXAMPLE 6 The method of Example 4 was followed using 20 volume equivale toluene in which the racemic diol is only partially soluble, and 1 'A equivalents of pig pancreatic lipase. The reaction was stirred for hours during which 47% of the racemic diol was converted (S)-es EXAMPLE 7 The method

of Example 4 was followed using 20 volume equivale tertiary butanol and 1 weight equivalent of pig pancreatic Hpase.

reaction mixture was stirred for 1.5 hours during which 32% of t racemic diol was converted to (S)-ester.

EXAMPLE 8 The method of Example 4 was followed using 4 volume equivalen vinyl butyrate. After 13 hours 52% of the racemic diol had been converted to the (S)-butyl ester.

EXAMPLE 9 The method of Example 4 was followed using 1 equivalent of pig pancreatic lipase. After 2.5 hours 50.6% of the racemic diol had been converted to (S)-ester.

EXAMPLE 10 The method of Example 9 was followed omitting the nitrogen stream.

After 4 hours 41.5% of the racemic chol had been converted to (S)-ester.

EXAMPLE 11 The method of Example 4 was followed using 10 volume equivalents of methyl isobutyl ketone and 4 volume equivalents of vinyl acetate. After 23 hours 47% of the racemic diol had been converted to (S)-ester.

EXAMPLE 12 The method of Example 11 was followed using a temperature of 800C.

After 4.5 hours 5196 of the racen-fic diol had been converted to (S)- ester.

The (R)-diol was isolated as described in Example 4 in 40% yield with greater than 97% enantiomeric excess.

EXAMPLE 13 The method of Example 4 was followed using 13 volume equivalents of m ethyl isobutyl ketone and 2.6 volume equivalents of vinyl acetate and 0.25 Weight equivalents of anhydrous sodium sulfate. After 3.75 hours 37% of the racemic diol had been converted to (S)-ester.

EXAMPLE 14 The method of Example 4 was followed using anhydrous methyliso- butylketone (H20 not measureable im. less than 0.05%) prepared by stirring reagent grade MlBK over calcium chloride and 1 weight equivalent of pig pancreatic lipase. After 2 hours 52.5% of the racemic diol had been converted to (S)-ester.

EXAMPLE 15 f The method of Example 4 was followed using reagent grade methyl isobutyl ketone (less than 0.5% H20) and 1 weight equivalent of pig pancreatic lipase. After 2.5 hours 52% of the racemic diol had been converted to (S)-ester.

EXAMPLE 16 The method of Example 4 was followed adding 0.1 weight % water to the anhydrous m ethyl isobutyl ketone. After 3 hours 52% of the racemic diol had been converted to iS)-ester.

EXAMPLE 17 The method of Example 4 was followed adding 0.5 weights % water to the anhydrous methylisobutylketone. After 5 hours 47.7% of the racemic diol had been converted to (S)-ester.

EXAMPLE 18 The method of Example 4 was followed adding 2 weight % water to the anhydrous methylisobut" ketone. After 3.5 hours 11.4% of the racernic diol had been converted to (S)-ester.

EXAMPLE 19 The method of Example 4 was followed using 1 weight equivalent of pig pancreatic lipase supported on 15 weight equivalents of inert support (see Table IV). The supported lipase was prepared by dissolving 1 weight equivalent enzyme in 20 weight equivalents 50mM potassium phosphate buffer pH 7.5 and adding 15 weight equivalents inert support. The suspensions were frozen at

-78OC: and Iyophilised at ambient temperature and 0.5mm Hg pressure over 2 days. 'The resulting dry solid was ground to homogeneqy and used directly in the reaction.

T aabWl e IVV Inert Support Time % Conversion Relative activity of (Hrs) racemic diol recycle catalyst Fine glass beads 2.5 10 - Fine washed sand 4.0 53 56 Celike 175 53 80 Neutral Alumina 2.5 33 33 None 2.5 51 26 EXAMPLE 20 The method of Example 4 was followed using 1 weight equivalent of pig pancreatic lipase supported on 0.05 weight equivalents of Eupergit (TIVI).

The supported lipase was prepared by dissolving 1 weight equivalent enzyme in 2.5 volume equivalents 50mM potassium phosphate buffer pH 7.5 and adding 0.05 weight equivalents Eupergit and stirring at ambient temperature for 72 hours. The solid was then filtered off and freeze dried as described in Example 19. The resulting dry solid was used directly in the reaction. After 2.5 hours 28% of the racemic diol had been converted to (S)-ester.

EXAMPLE 21 Synthesis of racemic (+ /-)-2-(2,4-difluoroi)henyl)-3-(1 H-1 .2,4- triazol-1 -YO propan-1,2-diol by recycle of (S)-ester.

1 weight equivalent of (S)-2-hydroxy-2-(2,4-difluorophenyl)-3-(1 H-1,2,4- triazol-1 -yl)propyl acetate was dissolved in 9 volume equivalents of toluene at 800C. 4 volume equivalents of 100TW caustic was added dropwise and the mixture stirred vigorously for 1 hour or until all the ester had been hydrolysed to diol as monitored by sampling from the organic phase by GLC. The mixture was cooled to 250C and 0.7 weight equivalents p-toluene sulfonyl chloride in 4 volume equivalents of toluene, with 0.2 weight equivalents of tetrabutyl ammonium bromide were added and the mixture stirred vigorously at ambient temperature until all the diol was converted to epoxide by GLC monitoring of the organic phase. The aqueous phase was separated and the Quene solution warmed to 600C 0.9 volume equivalents of 45% sulphuric acid was added dropwise and stirred for 30 minutes. The reaction mixture was cooled to 250C the organic layer was separated and the aqueous layer basified by addition of 50 volume equivalents of 1 M caustic to pl-19. To this solution 9 volume equivalents of MIBK were added. The solution was warmed to 650C and stirred then the aqueous layer salted with sodium chloride, stirred and separated. The MlBK solution was cooled and racemic diol crystallised out. The crystals were filtered, washed with cold MlBK and air dried.

This procedure gave 0.68 weight equivalents of diol, which by chiral HPLC was foud to be between 5-10% enantiomeric excess of (R)-diol.

EXAMPLE 22 Recycle of S-(+)-diol to raceMic diol S-(-P)-diol (1 equWalent) and p-toluene sulfonyl chloride (1 equivalent) are slurried together in 4 volume equivalents of toluene and stirred. 4M sodium hydroxide (2.5 volume equivalents) was added as drops over 30 minutes at ambient temperature. The reaction mixture was allowed to warm to 401C which was held for 30 minutes. The toluene layer was separated while the remaining aqueous layer was rewashed with a further 1 volume equivalent of toluene. The toluene extracts were combined and added sQwly over 10 minves to a solution of 45% (V:V) sulfuric acid (0.3 volume equivalents) at 650C. This temperature was held for a further 10 minutes and then cooled to 250C and basidified to pH 9 using 1 OOTw sodium hydroxide. 3 volume equivalents of water were added and the diol extracted from the aqueous solution by washing 3 times with 4 volume equivalents of ethyl acetate. The extracts were combined and the solvent removed under reduced pressure at 501C. The product yielded 0.75 equivalents of diol 53% (R)-enantiomer and 47% (S)- enantiorner.

EXAMPLE 23 Recycle of S(+)-diol to enriched R-diol S(+)-diol (1 equivalent), p-toluene sulfonVI chloride (1.1 equivalents) and tetra butVI am m oniurn bromide (0.031 equivalents) were slurried together in 4 volume equivalents of toluene at ambient temperature. The mixture was stirred vigorously and a solution of 4M sodium hydroxide (2.5 volume equivalents was added over 30 minutes. The solution was stirred a further 45 minutes until all the S(+)-diol had reacted. The total reaction mixture

was screened and the filter cake washed with toluene (0.5 volume equivalents). The lower aqueous phase from the filtrate was separated off and diluted with water (4 volume equivalents) then rewashed with 1.5 volume equivalents of toluene that has been used to wash the filter cake. This solution is then refiltered through celite to alloot separabon of the phases. The toluene layer is separated and all toluene fractions combined and then re-washed with water. The toluene layer is separated and the volume reduced to 3.5 volume equivalents.

Sulfuric acid (1.5M, 2.25 volume equivalents) at 250C is added to the toluene solution over 1 hour at 250C and stirred for 24 hours. The reaction mixture. was then hewed to 65 1 C for 2 hours, then cooled to room temperature. The aqueous layer was separated and the organic layer d4carded, The aqueous layer was basif ied with 100 Tw sodium hydroxide and the solution coded to 10*C from which the diol crystalfised out, was filtered off and air dried. 0.79 equivalents of diol were recovered which corresponded to 73.5% of R-diol and 26.5% of S- diol.

Claims

C LA IRMA S 1 Method of preparing an optically active compound of formula (1) CH2 R- C*-O h wherein R and R' are independently alkyl, alkenyl, alkynyl, alkoxy, alkoxyalkyl, cycloalkyl, aryl, aralkyl, a heterocyclic group or a Cj- C4 alkyl-heterocycie, each being optionally substituted, provided that R and R1 are not identical and * is an optically active chiral centre; the method comprising (a) treating a racemic compound of formula (11) 0 CH2-0-C-R2 R - K' (I wherein R and R' are as previously defined and R 2 is alkyl, aryl or aralkyl each optionally substituted, with a hydrolase; or (b) treating a racemic compound of formula (111) CH20H R - C - 0H h' M wherein R and R' are as previously defined, with a hydrolase in the presence of an acyl donor; and converting the optically active products of (a) and/or (b) to the optically active compound of formula (1).

2 Method of preparing an optically active compound of formula (11) or (111):

0 11 CH2-0-C-R 2 1 R - C - 0H CH20H I R- C-Ori wherein R and R' are independently alkyl, alkenyl, alkynyl, alkoxy, aikoxyalkyl, cycloalkyl, aryl, aralkyl, a heterocyclic group or a C,- C4 alkyl-heterocycle, each behg ophonally substituted, provided that R and R' are not identical and * is an optically active chiral centre; comprising treating the compound of formula (11) with a hydrolase or treating a compound of formula (111) with a hydrolase in the presence of an acyl donor, and optionally isolating the desired optically active ester or diol.

3 Method according to claim 1 or 2 wherein the hydrolase is a lipase, esterase, phosphatase, amidase, peptidase, sulphatase, **nitrilase (^)** or glycosidase.

4 Method according to claim 1, 2 or 3 wherein the hydrolase is a lipase or esterase.

Method according to any of claims 1 to 4 wherein the acyl donor is an alkyl ester or an alkenyl ester or an acid of formula RC02H where R is aikyl.

6 Method according to claim 5 wherein the acyl donor is an alkenyl ester.

7 Method according to any of claims 1 to 6 wherein the racemic diol of formula (111) is treated with a hydrolase in the presence of a solvent containing 0.5% or less by weight of water.

8 Method according to claim 7 wherein a hydrated or anydrous salt or mixtures thereof is added to the solvent.

9 Method according to any of claims 1 to 8 wherein the reacemic diol of formula (111) is treated with a hydrolase in the presence of a flow of nitrogen containing gas.

Method according to any of claims 1 to 9 wherein the treatment of the diol of formula (111) with a hydrolase is conducted at temperatures from 301C to 800C.

Method according to any of claims 1 to 10 in which the optically active dioi of formula (111) is isolated and either (a) the organic solution is concentrated and seeded with optically active diol of formula (111) to selectively crystallise the optically active diol of formula (111) from the mixture, or (b) the organic solvent is removed and the resultant oil triturated with a different organic solvent from which the desired optically active diol of formula (111) is crystallised out from the mixture.

12 Method according to claim 11 in which the different organic solvent is dichloromethane.

13 Method according to any of claims 1 to 12 wherein after the hydrolase treatement there is an additional step of recycling the unwanted optically active ester or diol to the corresponding racemic diol of formula (111).

14 Method according to claim 13 wherein the unwanted optically active ester of formula (11) is recycled to the corresponding racernic Sol of formula (111) by hydrolysing the optically active ester of formula (11) by adding an aqueous base and either isolati the corresponding diol of formula (111) or treating the correspondir diol in the aqueous base with an optionally substituted aryl or alk sulphonyl halide optionally in the presence of a phase transfer catalyst to form the corresponding optically active epoxide of formula (1) and hydrolysing the optically active epoxide with an aqueous acid to give the racemic diol of formula (111).

Method according to claim 13 wherein the unwanted optically active diol of formula (111) is recycled to the racemic diol of formul (111) by reacting the optically active diol of formula (111) with an ar) or alkyl sulfonyl halide in a solvent and optionally in the presence of a phase transfer catalyst to form the corresponding optically active epoxide of formula (1) and hydrolysing the optically active epoxide with an aqueous acid to give the racemic diol of formula 0 11).

16 Method according to any of claims 1 to 15 wherein R and R1 are independently, phenyk benzyk C.7 cyc1oalkyl, Q0 alkyl,C1.10 alkenyl, C1.10 alkynyl, C1.10 alkoxy, C1.10 alkoxyalkyl, 1,2,4- triazole, -CH2-1,2,4-triazole, 1,3,4-triazole, imidazole, pyrimidine, pyrazine, oxazole or pymzde, each optionally substituted.

17 Method for pmparaton of compounds of formula V:

OR I Xn C'- R5 I I UM2 I N-N Y N M wherein R' is alkyl, alkenyl, alkynyl, alkoxy, alkoxyalkyl, cycloalkyl, aryl, aralkyl, a heterocyclic group or a Cl-C4 alkyl- heterocycle, Y is an optional substituent, X is halogen, alkyl, alkoxy, haloalkyl, haloalkoxy, cyano, nitro, amino, hydroxy, sulphonyl or phenyl and n is 0 to 5, which comprises the steps of:

(i) preparing a compound of formula (IV):

CH2 x" C*- 0 1 1 U"2 I N-N Ll N 'J(IV) wherein X and n are as previously defined, by (a) treating a compound of formula 0 11 CH2-O-C-C3H7 I x C-OH ' I I:D I I UM2 I N-N ~1 NJ wherein X and n are as previously defined, with a hydrolase; or (b) treating a compound of formula CH20H U-M Xr, CH2 N-N Li N J- wherein X and n are as previously defined, with a hydrolase in the presence of an acyl donor; (c) converting the optically active products of (a) and (b) to the optically active epoxide of formula OW Oi) converting the optically active epoxide of formula (IV) to the optically active compound of formula (V) in an organic solvent in the presence of a base.

is Use (^) of hydroiase for the prepambon of an ophcally active ester of formula (11) or an optically active dio.1 of formula (111) :

0 11 - OH CH20H I R - C- OH wherein R and R' are independently alkyl, alkenyl, alkynyl, alkoxy, alkoxyalkyl, cycloalkyl, aryl, aralkyl, a heterocyclic group or a Cj- C, alkyl-heterocycle, each bQng optionally substituted, provided that Ft and 11 are not identical and * is an optically active chiral centre.

19 Method or use (^) according to any of claims 1 to 16 and 18 wherein R is phenyl or halophenyl and R' is aC1-C4 alkyl- 1, 2,4-triazole.

Compound of formula 0 CH2-0-C-R 2 Xr, C-OH CH2 N-N 11, N J and enanhome6c forms thereof, wherein R 2 is alkyl, X is halogen, alkyl, haloalkyl, alkoxy, haloalkoxy, cyano, nitro, amine, hydroxy, sulphonyl or phenyl; and n is 0 to 5.

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